

61 (a) generating a three dimensional model of a parent alpha-amylase structure, utilizing data from Appendix 1 and a computer programmed for generating said model from said data;

I (b) identifying in said three-dimensional parent alpha-amylase structure generated in step (a) at least one amino acid residue or at least one structural part; wherein an alteration in said at least one amino acid residue or said at least one structural part is predicted to result in an altered property, <sup>said</sup> and wherein said altered property is selected from the group consisting of substrate specificity, substrate binding, substrate cleavage pattern, temperature stability, pH dependence of enzymatic activity, pH dependence of stability, stability towards oxidation,  $Ca^{2+}$  dependency and specific activity;

(c) modifying the sequence of a nucleic acid encoding said parent alpha-amylase to produce a nucleic acid encoding a deletion, insertion, or substitution of one or more amino acids at a position corresponding to said at least one amino acid residue or at least one structural part identified in step (b); and

(d) expressing the modified nucleic acid of step (c) in a host cell to produce said variant alpha amylase.

62 283. (amended) The method according to claim 81, wherein said three-dimensional alpha amylase structure has an A domain, a B domain and a C domain, wherein said A domain has an amino acid sequence corresponding to residues 1-103 and 206-395 of SEQ ID NO:2; said B domain has an amino acid sequence corresponding to residues 104-205 of SEQ ID NO:2 and said C domain has an amino acid sequence corresponding to residues 396-483 of SEQ ID NO:2.

63 86. (amended) A method for producing a variant of a parent alpha-amylase having an altered property relative to said parent alpha-amylase, wherein said altered property is selected from the group consisting of substrate specificity, substrate binding, substrate cleavage pattern, temperature stability, pH dependence of enzymatic activity, pH

63 dependence of stability, stability towards oxidation,  $\text{Ca}^{2+}$ -dependency and specific activity, wherein said parent alpha-amylase has a sequence of at least 70% homology to the sequence of SEQ ID No: 13, when 'homology' is determined by the GAP program (Genetic Computer Group, Version 7.8) using default values for GAP penalties, said method comprising

(a) generating a model of a three dimensional structure of a parent alpha-amylase using a computer programmed for generating a model structure and atomic coordinates shown in Appendix 1;

(b) utilizing said model generated in step (a) and modeling methods to identify at least one amino acid residue or at least one structural part; wherein an alteration in said at least one amino acid residue or said at least one structural part is predicted to result in an altered property, and wherein said altered property is selected from the group consisting of substrate specificity, substrate binding, substrate cleavage pattern, temperature stability, pH dependence of enzymatic activity, pH dependence of stability, stability towards oxidation,  $\text{Ca}^{2+}$ -dependency and specific activity;

(c) modifying the sequence of a nucleic acid encoding said parent alpha-amylase to produce a nucleic acid encoding a deletion, insertion, or substitution of one or more amino acids at a position corresponding to said at least one amino acid residue or at least one structural part identified in step (b); and

(d) expressing the modified nucleic acid of step (c) in a host cell to produce said variant alpha-amylase.

Please add the following new claims 87-92:

64 87. (new) A method for producing a variant of a parent alpha-amylase having an altered property relative to said parent alpha-amylase, said parent alpha-amylase comprising an active site residue, said method comprising

sub H2 (a) generating a model of a three dimensional structure of a parent alpha-amylase, wherein said three-dimensional alpha amylase structure comprises calcium and sodium ions defined by atomic coordinates of Appendix 1;

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(b) utilizing said three dimensional structure generated in step(a) and modeling methods to identify in said parent alpha-amylase structure at least one amino acid residue or structural part within 10Å of calcium or sodium ions;

(c) modifying the sequence of a nucleic acid encoding said parent alpha-amylase to produce a nucleic acid encoding a deletion, insertion, or substitution of one or more amino acids at a position corresponding to said at least one amino acid residue identified in step (b); and

(d) expressing the modified nucleic acid in a host cell to produce said variant alpha amylase.

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88. (new) The method according to claim 87, wherein the variant has a decreased calcium ion dependency of enzymatic activity or stability.

89. (new) A method for producing a variant of a parent alpha-amylase having an altered property relative to said parent alpha-amylase, said parent alpha-amylase comprising an active site residue, said method comprising

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(a) generating a model of a three dimensional structure of a parent alpha-amylase, wherein said three-dimensional alpha amylase structure comprises an active site residue defined by atomic coordinates of Appendix 1;

(b) utilizing said three dimensional structure generated in step (a) and modeling methods to identify in said parent alpha-amylase structure at least one amino acid residue or structural part within 15Å from said active site residue;

(c) modifying the sequence of a nucleic acid encoding said parent alpha-amylase to produce a nucleic acid encoding a deletion, insertion, or substitution of one or more amino acids at a position corresponding to said at least one amino acid residue identified in step (b); and

(d) expressing the modified nucleic acid in a host cell to produce said variant alpha amylase.

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90. (new) The method according to claim <sup>5</sup>89, wherein the variant has an altered pH optimum dependence of enzymatic activity relative to the parent.

91. (new) A method for producing a variant of a parent alpha-amylase having an altered property relative to said parent alpha-amylase, said parent alpha-amylase comprising an active site residue, said method comprising

(a) generating a model of a three dimensional structure of a parent alpha-amylase, wherein said three-dimensional alpha amylase structure comprises a substrate binding area defined by atomic coordinates of Appendix 1;

(b) utilizing said three dimensional structure generated in step (a) and modeling methods to identify in said parent alpha-amylase structure the substrate binding area;

(c) modifying the sequence of a nucleic acid encoding said parent alpha-amylase to produce a nucleic acid encoding a deletion, insertion, or substitution of one or more amino acids at a position corresponding to said at least one amino acid residue identified in step (b); and

(d) expressing the modified nucleic acid in a host cell to produce said variant alpha amylase.

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92. (new) The method according to claim <sup>7</sup>91, wherein the variant has a reduced ability to cleave a substrate close to a branching point relative to the parent.

#### REMARKS

Claims 81-86 are pending in the above referenced application. Claims 84 and 85 have been canceled. Claims 81, 83 and 86 have been amended to more distinctly claim that which Applicants regard as their invention.

New claims 87-92 have been added to recited specific embodiments. Specifically, in new claims 87-88, at least one amino acid residue or structural part within 10Å of calcium or sodium ions is identified in said parent alpha-amylase structure (claim 87, step (b)); in new claims 89-90, at least one amino acid residue or structural part within 15Å